

Appendix E

Correlation of Standard Methods Chlorophyll Quantification and In-vivo Chlorophyll Fluorescence Measurements

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Introduction

The San Joaquin River in the Central Valley is a highly impaired water body and numerous studies are being conducted investigating the impact of diffuse pollution on water quality and habitat in this region (Stringfellow, 2008). One component of the larger water quality picture is the source and quantity of phytoplankton in this system. It has been well established that quantification of photosynthetic pigments in water samples is a reasonable method of estimating phytoplankton biomass because chlorophyll *a*, the main pigment present in green plants, has been shown to be between 1-2% of the dry weight of planktonic algae (Clesceri et al, 1998). The aim of this report is to analyze the relationship between two methods for measuring chlorophyll *a* concentrations, extraction and spectrophotometric determination of chlorophyll *a* content and fluorometric measurement in the field. Analysis was conducted on the data sets from each year, for all three years compiled, and for the different YSI units used during 2005 through 2007 by the Environmental Engineering Research Program (EERP).

Methods

Standard Methods for the Examination of Water and Wastewater section 10200 H. (Clesceri et. al., 1998) as well as the *EERP Lab Protocol Book* (Borglin et. al., 2008) and *EERP Field Protocol Book* (Hanlon et. al., 2008) describes the extraction and spectrophotometric quantification process used to determine chlorophyll *a* concentrations and thus relative phytoplankton loads in the San Joaquin River watershed by EERP for the years 2005 through 2007. In addition to extraction and spectrophotometric quantification of chlorophyll pigments from grab samples, a YSI Sonde 6600 with 6025 chlorophyll sensor was used at every grab sample site to fluorometrically measure and record a corresponding chlorophyll *a in-vivo* value during that same period, 2005-2007.

Results

Extracted chlorophyll *a* concentration in mg/L when compared to YSI Sonde 6600 fluorescence measurement had an r^2 value of 0.858 and a slope of 8.818 (where $n = 370$) in 2005, an r^2 value of 0.820 and a slope of 7.999 (where $n = 504$) in 2006, an r^2 value of 0.773 and a slope of 9.140 (where $n = 702$) in 2007, and an r^2 value of 0.7996 and a slope of 8.753 (where $n = 1576$) for 2005-2007 (Figures 1-4). For EERP Sonde no. 1 (serial no. 04M1920 AA), the chlorophyll *a* concentration versus Sonde fluorescence yielded an r^2 value of 0.814 and a slope of 8.634 (where $n = 247$) in 2006, an r^2 value of 0.725 a slope of 8.469 (where $n = 432$) in 2007, and an r^2 value of 0.776 a slope of 8.592 (where $n = 1049$) in for 2005-2007. For EERP Sonde no. 2 (serial no. 05B1294 AA), the chlorophyll *a* concentration versus Sonde fluorescence yielded an r^2 value of 0.873 and a slope of 6.918 (where $n = 257$) in 2006, an r^2 value of 0.914 a slope of 11.090 (where $n = 270$) in 2007, and a r^2 value of 0.861 a slope of 9.259 (where $n = 527$) for 2005-2007 (Figures 5-10). During the 2005 sampling year only EERP Sonde no. 1 (serial no. 04M1920 AA) was used. See Table 1 for results.

Discussion

Over the research period of 2005-2007 the slope between corresponding YSI Sonde fluorescence readings and chlorophyll *a* extract values remained relatively consistent, between 7.0 and 9.3. The value 8.8 was used to correct fluorescence to chlorophyll *a* concentration in reported electronic data sets. The variation in these values from year to year could be attributed to variations in each years range of chlorophyll concentrations due to the extremely wet conditions of 2006 and the drought-like conditions of 2007, which influence residence times and algal growth thus causing chlorophyll *a* levels to fluctuate to the extremes of both methods' valid ranges. This trend from 2005 through 2007 is also evidenced by the range of values from year to year (see Figures 1-3).

There is a decrease in r^2 values which tracks the total number of samples taken, from 304 in 2005 (r^2 value of 0.858), to 504 in 2006 (r^2 value of 0.820), and finally 702 in 2007 (r^2 value of 0.773). Some of the discrepancies between the values generated by these two methods can be explained by the inability of the fluorometric method to differentiate between healthy living chlorophyll *a* and one of its major degradation products, pheophytin, as well as the problems affiliated with the consistent handling and analysis of highly unstable compounds, such as chlorophyll *a*, as is the case with the standard methods extraction and quantification process.

Examination of the slope and r^2 value differences between YSI Sonde units 1 and 2 shows further the dissimilarity in sample ranges between the two last sampling years, 2006 and 2007, due to the differing weather conditions (Figures 5 and 7). The variation in relationships (slopes) between the two methods for each unit can be explained to some extent by the disparity in sample sites routinely measured by each Sonde unit. Sample sites vary greatly in accessibility and water quality and many sites prove problematic for getting accurate fluorometric and laboratory measurements due to heterogeneous chlorophyll concentrations, the particulate nature of chlorophyll, high pheophytin concentrations, and very shallow water columns. These problematic sites were not evenly divided between the two units and because the same Sondes were used in the same routine locations, those problem sites may have influenced the evident relationships between methods. In the future, rotating sondes between sampling crews is recommended to provide a better comparison between sondes.

References

- American Public Health Association, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.
- Stringfellow, W.T., 2008. Progress Report: Discharge Management Program Monitoring and Evaluation - West Stanislaus County. Stockton, CA.
- Borglin, S.E., Burks, R.D., Hanlon, J.S., Stringfellow, W.T., 2008. EERP Lab Protocol Book. University of the Pacific, Stockton, CA.

YSI Environmental Operations Manual, 2005. 6-Series Environmental Monitoring Systems, Yellow Springs, OH.

Table 1: R-squared and slope results for years 2005-2007

Year	Sonde 1 (no. 04M1920 AA)			Sonde 2 (no. 05B1294 AA)			Over All		
	<i>n</i>	R-Squared Value	Slope	<i>n</i>	R-Squared Value	Slope	<i>n</i>	R-Squared Value	Slope
2005	370	0.858	8.818				370	0.858	8.818
2006	247	0.814	8.634	257	0.873	6.918	504	0.82	7.999
2007	432	0.725	8.469	270	0.914	11.09	702	0.773	9.14
2005-2007	1049	0.776	8.592	527	0.861	9.259	1576	0.7996	8.753

Figure 1: 2005 Sonde fluorescence vs. chlorophyll *a* concentration (SM TC Method).

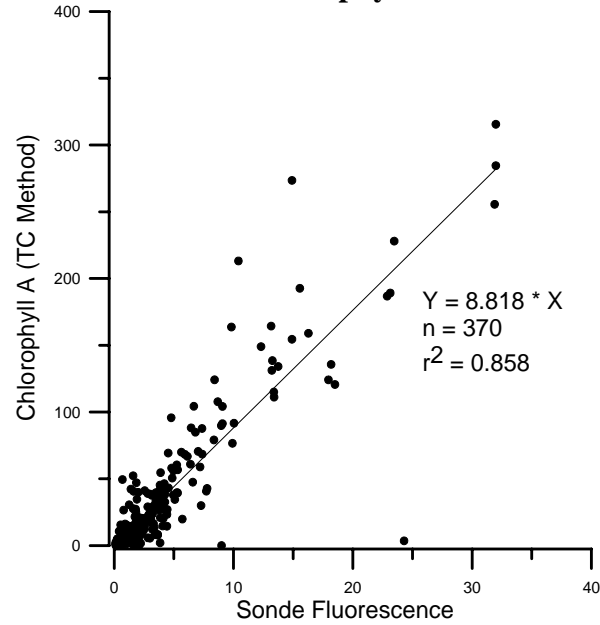


Figure 2: 2006 Sonde fluorescence vs. chlorophyll *a* concentration (SM TC Method).

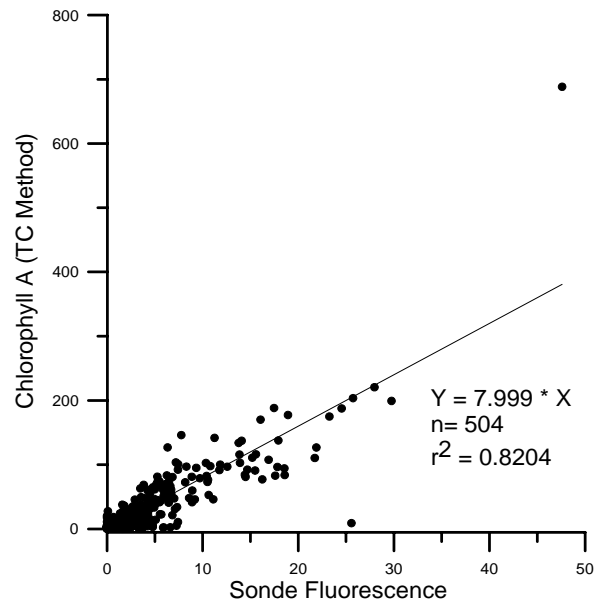


Figure 3: 2007 Sonde fluorescence vs. chlorophyll *a* concentration (SM TC Method).

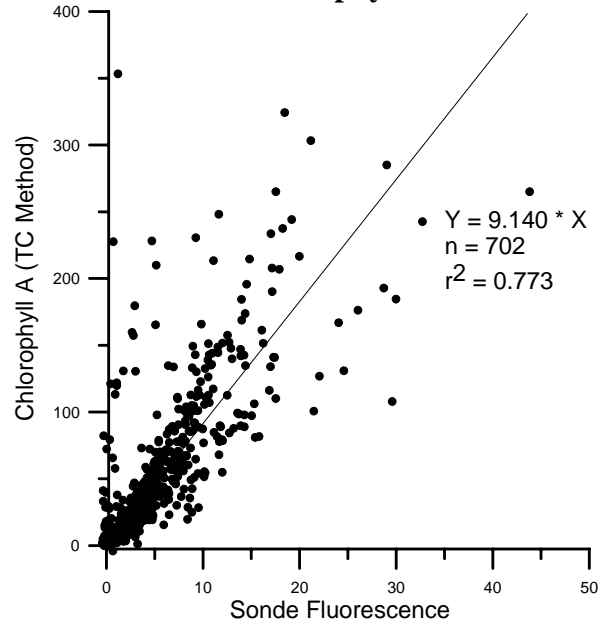


Figure 4: 2005 - 2007 Sonde fluorescence vs. chlorophyll *a* concentration (SM TC Method).

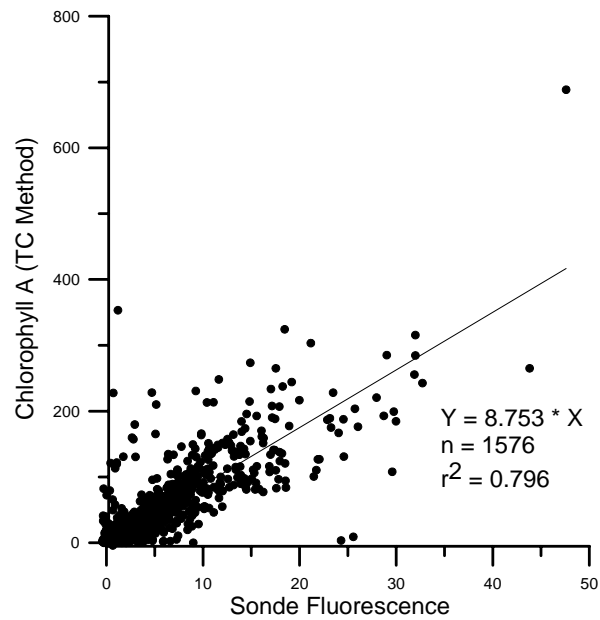


Figure 5: 2006 Sonde No. 1 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

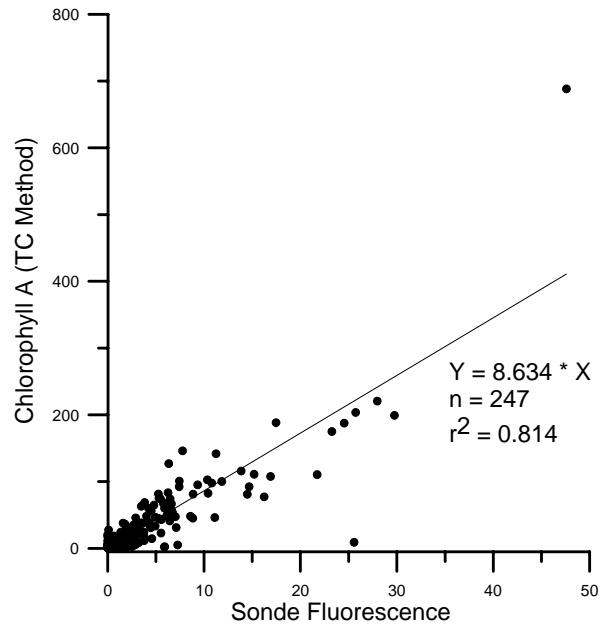


Figure 6: 2006 Sonde No. 2 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

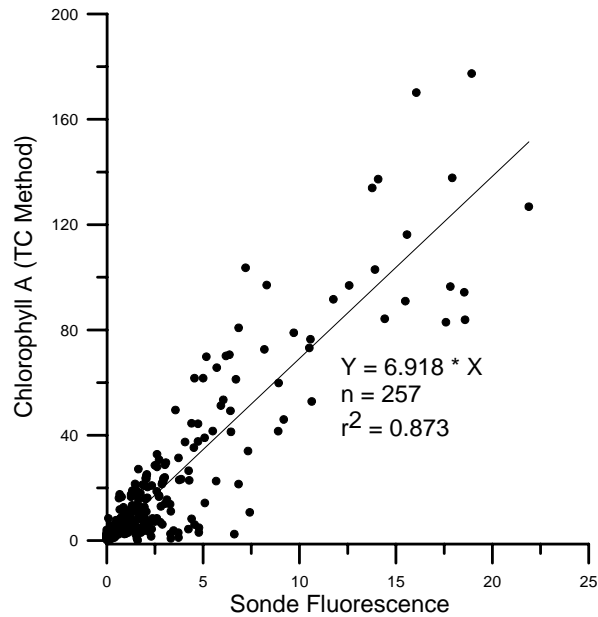


Figure 7: 2007 Sonde No. 1 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

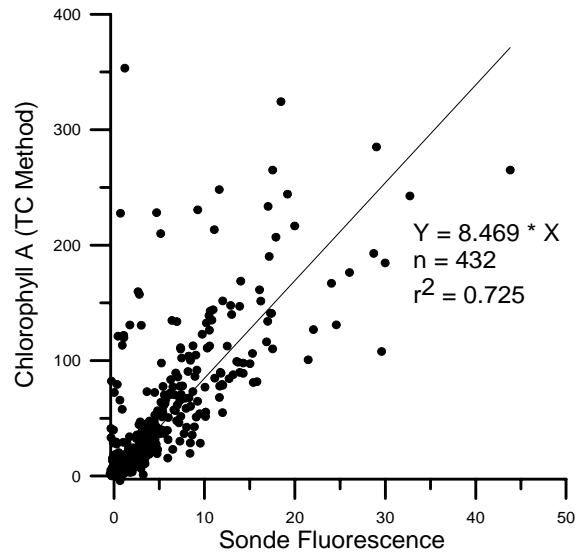


Figure 8: 2007 Sonde No. 2 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

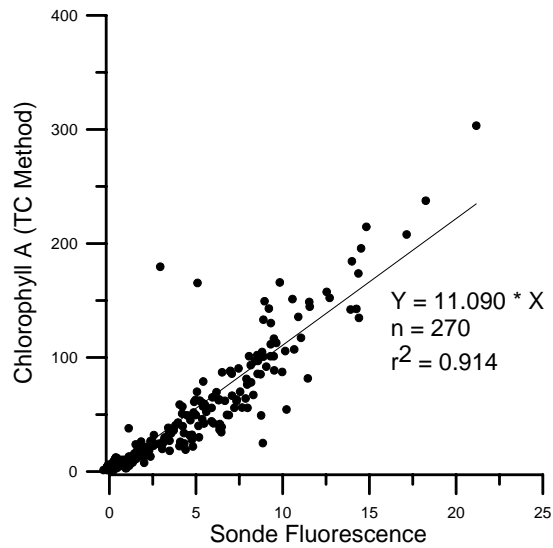


Figure 9: 2005 - 2007 Sonde No. 1 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

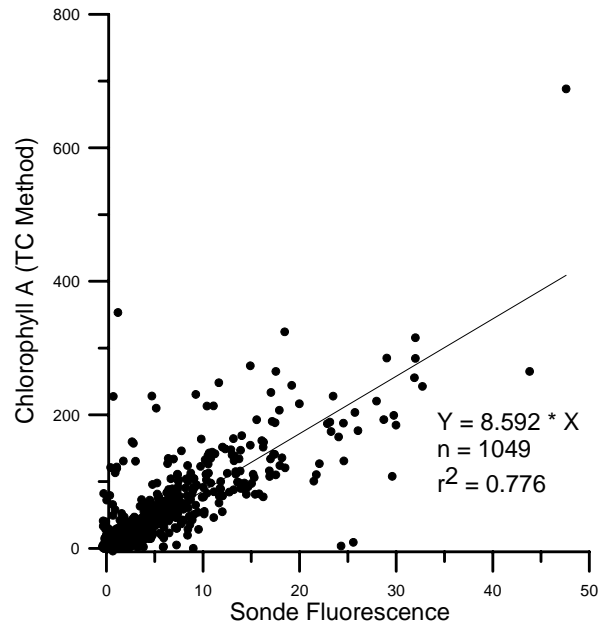


Figure 10: 2005 - 2007 Sonde No. 2 fluorescence vs. chlorophyll *a* concentration (SM TC Method).

